(12) UK Patent Application (19) GB (11) 2 314 856 (13) A

(43) Date of A Publication 14.01.1998

(21) Application No 9713991.9

(22) Date of Filing 03.07.1997

(30) Priority Data
(31) 9613957 (32) 03.07.1996 (33) GB

(71) Applicant(s)
British Textile & Technology Group

(Incorporated in the United Kingdom)

Shirley House, Wilmslow Road, Didsbury, MANCHESTER, M20 2RB, United Kingdom

The University of Leeds

(Incorporated in the United Kingdom)

175 Woodhouse Lane, LEEDS, LS2 9JT, United Kingdom

(72) and (74) continued overleaf

(51) INT CL[6]
D21H 17/24

(52) UK CL (Edition P)
D1R RDC R110 R307 R461 R647
U1S S1049

(56) Documents Cited
GB 2248639 A EP 0393825 A
WPI Abstract Accession No. 93-270126/199334 & JP 05186945 A (FUJI) 27.07.93 (see abstract) WPI Abstract Accession No. 93-408355/199351 & JP 05305129 A (A. IND. SCI.) 19.11.93 (see abstract) WPI Abstract Accession No. 93-392671/199349 & JP 5295005 A (NAKANO) 09.11.93 (see abstract)

(58) Field of Search
UK CL (Edition O) D1R
INT CL[6] D21H
Online:WPI

(54) Cellulose Products

(57) Cellulose, which may be microbially produced, is treated with chitosan to form, e.g. medical dressings or pads. The cellulose may be immersed in a chitosan solution, e.g. chitosan dissolved in an acid.

GB 2 314 856 A

(72) Inventor(s)
Paul Frederick Hamlyn
John Cright n
Michael George Dobb
Alexander Tasker

(74) Agent and/or Address for Service
McNeight & Lawrence
Regent House, Heaton Lane, STOCKPORT, Cheshire,
SK4 1BS, United Kingdom

NSDOCID: <GB 2314856A I >

CELLULOSE PRODUCT

This invention provides novel cellulose products.

Cellulose is known in various forms and produced by various methods in fibre form. In some forms it is used in medical e.g. wound dressings. One such form, disclosed in GB-2 131 701-A is a pellicle of microbially-produced cellulose obtained by culturing Acetobacter bacteria.

The microbially-produced pellicle has very fine cellulose fibres and can be highly absorbent, for example of wound exudate, or can remain moist for considerable periods of time, and, by virtue of evaporation, can have a cooling effect useful in treating burns.

The present invention enhances the properties of cellulose, especially microfibrillar cellulose such as is microbially produced, and in particular enhances its properties in medical and particularly wound dressings.

The invention comprises cellulose treated with chitosan.

The cellulose can be microfribillar and in particular microbially-produced cellulose, more particularly never-dried, microbially-produced cellulose, and the invention comprises medical dressings or pads, e.g. liquid-soaked for medical applications made therefrom.

The chitosan content by weight may be of the order of 100 / of the weight of the cellulose weight.

In addition, the dressing or pad can be treated with a medication, and may be supported or reinforced with a supporting or reinforcing backing.

The dressing or pad may be packed, wet, in an impermeable package, and may be packed with a reduced liquid loading (as by pressing liquid out from it) permitting further absorption of liquid e.g. of wound exudate.

The invention also comprises a method for making a chitosan-treated cellulose fibre product comprising immersing the cellulose in a solution of chitosan.

The chitosan may be dissolved in an acid such as acetic and/or citric acid, which may be in 1-6% solution, preferably 2%, the chitosan solution being a 1-4 % preferably 2% solution. The cellulose may be immersed for at least one hour, preferably 3 hours in the chitosan solution, and the immersion may proceed at room temperature.

The cellulose may be drip-dried after the immersion and washed, washing preferably being repeated or continued, in distilled water, until the pH is 7, and it may then be sterilised, e.g. autoclaved.

While any fibrous cellulose product can be treated according to the invention, the advantages are perhaps most appreciated when the cellulose is a

microbially-produced, never-dried pellicle. GB-2 131 701-A suggests using a particular Acetobacter bacteria, *A.xylinium*; *A.pasteurianus* can also be used to good effect.

The bacteria may be cultured in liquid nutrient medium at an initial pH of between 5.5 and 7 and at a temperature between 15 and 25°C, preferably at an initial pH of 7 and a temperature of 29°C ± 1°C.

The liquid nutrient medium may comprise glucose (20g), peptone (5g), yeast extract (5g), sodium dihydrogen phosphate (2.7g) and citric acid (1.15g) in 1 litre distilled water.

The pellicle should be cultured, for best results, in a substantially morticulen culturing medium, the time of cultivation being dependent on the thickness of fleece or pad required - several hours to several days, typically.

The harvested pellicle may, after drawing and pressing out much of its water, be soaked in aqueous sodium hydroxide to remove the bacteria, then immersed in an acid such as hydrochloric acid to neutralise the alkali.

Production of a never-dried pellicle of bacterial cellulose and incorporation of chitosan will now be described.

Acetobacter bacteria (preferably *A.pasteurianus*) are cultured in liquid nutrient medium as reported by Schramm and Hestrim (J. Biochem., 58, 1954) at an initial pH of between 5.5 and 7.0 (preferably 7.0) and at a temperature of between 15 and

35°C, preferably 29 ± 1°C. The recipe for the medium is 20.0g glucose, 5.0g peptone, 5.0g yeast extract, 2.70g sodium dihydrogen phosphate, 1.15g citric acid and 1 litre distilled water.

A culture tray (100mm x 200mm) is filled with 500ml of the culture medium, then covered with an autoclavable bag and sterilised. To the sterile medium are added 200 microlitres of inoculum from a previously inoculated stock flask (2-3 days old) and the tray placed in an unlit incubator at 29 ± 1°C and left undisturbed for 14 days.

The resulting pellicle is harvested from the tray and gently pressed between absorbent sheets to expel about 80% of its liquid content before soaking in 3% aqueous sodium hydroxide solution for 12 hours to remove the bacteria. The pellicle is pressed again and the soaking and pressing repeated twice. The pellicle is then immersed in 3 / aqueous hydrochloric acid for 3 hours to neutralise the alkali followed by pressing between absorbent sheets to remove about 80% of the liquid.

The pellicle is then transferred to a bath of distilled water and repeatedly pressed and allowed to reabsorb fresh water until the pH is about 7.0 and all the sodium chloride salt removed.

The clean pellicle can be stored temporarily in distilled water or, for longer periods, in ethanol at a temperature between 0 and 5°C.

A never-dried pellicle thus obtained is immersed in a 1-4%, preferably 2% solution of chitosan in acetic and/or citric acid (1-6%, preferably 2%) for 3 hours at room

temperature (16-25°C, preferably 20°C). The pellicle is then removed and allowed to drip-dry for at least 10 minutes and then washed in distilled water for 2 hours. The washing process is repeated at least twice or until the pH is about 7.0.

The chitosan-treated cellulose pellicle is then sterilised in an autocalve, in autocavable, sealable bags, for 20 minutes at 120°C, 2-3 atmospheres pressure. Once sterilised the bag can be sealed and stored at a temperature between 0 and 5°C.

The chitosan-treated pellicle can be used for all of the purposes mentioned in GB-2 131 701-A with enhanced wound healing properties.

CLAIMS

1. Cellulose treated with chitosan.

2. Microfribrillar cellulose treated with chitosan.

3. Microbially-produced cellulose treated with chitosan.

4. Never-dried microbially-produced cellulose treated with chitosan.

5. A medical dressing made from cellulose treated with chitosan according to any one of claims 1 to 4.

6. A pad for medical applications comprising a sterile, liquid-soaked pellicle of microbially-produced cellulose, treated with chitosan.

7. A dressing or pad according to claim 5 or claim 6, of which the chitosan content by weight is of the order of 100% of the weight of the cellulose weight.

8. A dressing or pad according to any one of claims 5 to 7, treated with a medication.

9. A dressing or pad according to any one of claims 5 to 7, supported or reinforced with a supporting or reinforcing backing.

10. A dressing or pad according to any one of claims 5 to 9, packed, wet, in an impermeable package.

11. A dressing or pad according to claim 10, packed with a reduced liquid loading permitting further absorption of liquid e.g. of wound exudate.

12. A method for making a chitosan-treated cellulose fibre product comprising immersing the cellulose in a solution of chitosan.

13. A method according to claim 12, in which the chitosan is dissolved in an acid.

14. A method according to claim 13, in which the acid is acetic and/or citric acid.

15. A method according to claim 14, in which the acid is a 1-6% solution.

16. A method according to claim 15, in which the acid is a 2% solution.

17. A method according to any one of claims 13 to 16, in which the chitosan solution is a 1-4% solution.

18. A method according to claim 17, in which the chitosan solution is a 2% solution.

19. A method according to any one of claims 12 to 18, in which the cellulose is immersed for at least one hour, preferably 3 hours in the chitosan solution.

20. A method according to any one of claims 12 to 18, in which the immersion proceeds at room temperature.

21. A method according to any one of claims 12 to 20, in which the cellulose is drip-dried after the immersion.

22. A method according to any one of claims 12 to 21, in which the cellulose is washed after the immersion.

23. A method according to claim 22, in which the cellulose is repeatedly washed after immersion.

24. A method according to claim 22 or claim 23, in which the washing is effected in distilled water.

25. A method according to any one of claims 22 to 24, in which washing is continued until the pH is 7.

26. A method according to any one of claims 12 to 25, in which the treated cellulose is sterilised.

27. A method according to claim 26, in which the treated cellulose is autoclaved.

28. A method according to any one of claims 12 to 27, in which the cellulose fibre product is a microbially-produced, never-dried pellicle.

29. A method according to claim 28, in which the pellicle is prepared from Acetobacter bacteria.

30. A method according to claim 29, in which the Acetobacter bacteria comprise Acetobacter pasteurianus.

31. A method according to claim 29 or claim 30, in which the bacteria are cultured in liquid nutrient medium at an initial pH of between 5.5 and 7 and at a temperature between 15 and 35°C.

32. A method according to claim 31, in which the initial pH is 7 and the temperature is 29°C ± 1°C.

33. A method according to claim 31 or claim 32, in which the liquid nutrient medium comprises glucose (20g), peptone (5g), yeast extract (5g), sodium dilydrogen phosphate (2.7g) citric acid (1.15g) and 1 litre distilled water.

34. A method according to any one of claims 28 to 33, in which the pellicle is cultured in a substantially motionless culturing medium.

35. A method according to any one of claims 28 to 34, in which the harvested pellicle is soaked in aqueous sodium hydroxide to remove the bacteria.

36. A method according to claim 35, in which the pellicle is immersed in an acid such as hydrochloric acid to neutralise the alkali.

Amendments to the claims have been filed as follows

1. A pad for medical applications comprising a sterile, liquid-soaked pellicle of microbially-produced cellulose, treated with chitosan.

2. A pad according to claim 1, of which the chitosan content by weight is of the order of 100% of the weight of the cellulose weight.

3. A pad according to either one of claims 1 or 2, treated with a medication.

4. A pad according to either one of claims 1 or 2, supported or reinforced with a supporting or reinforcing backing.

5. A pad according to any one of claims 1 to 4, packed, wet, in an impermeable package.

6. A pad according to claim 5, packed with a reduced liquid loading permitting further absorption of liquid e.g. of wound exudate.

7. A method for making a chitosan-treated cellulose fibre product comprising immersing the cellulose in a solution of chitosan, the chitosan being dissolved in an acid.

8. A method according to claim 7, in which the acid is acetic and/or citric acid.

9. A method according to claim 8, in which the acid is a 1-6% solution.

10. A method according to claim 9, in which the acid is a 2% solution.

11. A method according to any one of claims 7 to 10, in which the chitosan solution is a 1-4% solution.

12. A method according to claim 11, in which the chitosan solution is a 2% solution.

13. A method according to any one of claims 7 to 12, in which the cellulose is immersed for at least one hour, preferably 3 hours in the chitosan solution.

14. A method according to any one of claims 7 to 13, in which the immersion proceeds at room temperature.

15. A method according to any one of claims 7 to 14, in which the cellulose is drip-dried after the immersion.

16. A method according to any one of claims 7 to 15, in which the cellulose is washed after the immersion.

17. A method according to claim 16, in which the cellulose is repeatedly washed after immersion.

18. A method according to claim 16 or claim 17, in which the washing is effected in distilled water.

19. A method according to any one of claims 16 to 18, in which washing is continued until the pH is 7.

20. A method according to any one of claims 7 to 19, in which the treated cellulose is sterilised.

21. A method according to claim 20, in which the treated cellulose is autoclaved.

22. A method according to any one of claims 7 to 21, in which the cellulose fibre product is a microbially-produced, never-dried pellicle.

23. A method according to claim 22, in which the pellicle is prepared from Acetobacter bacteria.

24. A method according to claim 23, in which the Acetobacter bacteria comprise Acetobacter pasteurianus.

25. A method according to claim 23 or claim 24, in which the bacteria are cultured in liquid nutrient medium at an initial pH of between 5.5 and 7 and at a temperature between 15 and 35°C.

26. A method according to claim 25, in which the initial pH is 7 and the temperature is 29°C ± 1°C.

27. A method according to claim 25 or claim 26, in which the liquid nutrient medium comprises glucose (20g), peptone (5g), yeast extract (5g), sodium dilydrogen phosphate (2.7g) citric acid (1.15g) and 1 litre distilled water.

28. A method according to any one of claims 22 to 27, in which the pellicle is cultured in a substantially motionless culturing medium.

29. A method according to any one of claims 22 to 28, in which the harvested pellicle is soaked in aqueous sodium hydroxide to remove the bacteria.

30. A method according to claim 29, in which the pellicle is immersed in an acid such as hydrochloric acid to neutralise the alkali.

The Patent Office

15

Application No: GB 9713991.9 **Examiner:** G WERRETT
Claims searched: 1-36 **Date of search:** 17 September 1997

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.O): D1R.

Int Cl (Ed.6): D21H.

Other: Online:WPI.

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X	GB 2248639 A (D.G.A. IND. SCI.)	1,2,3,12.
X	EP 0393825 A1 (D.G.A. IND. SCI.) see p. 2, l. 6, p. 3, l. 14.	1,2,12.
X	WPI Abstract Accession No. 93-270126/199334 & JP 05186945 A (FUJI) 27.07.93 (see abstract).	1, 5.
X	WPI Abstract Accession No. 93-408355/199351 & JP 05305129 A (A. IND. SCI.) 19.11.93 (see abstract).	1, 5, 12.
X	WPI Abstract Accesion No. 93-392671/199349 & JP 5295005 A (NAKANO) 09.11.93 (see abstract)	1-4, 12.

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.